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Human Diseases Associated with Mycoplasmas

With an Appendix on Simple Culture Techniques

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■ *The mycoplasmas (formerly called pleuropneumonia-like organisms, or* PPLO*) are a group of pleomorphic micro-organisms characterized by lack of cell wall and ability to form colonies on agar resembling tiny fried eggs. They have been recognized as pathogens of lower mammals since 1898. Of the more than 40 known veterinary species, many are pathogens, commonly causing pneumonia, arthritis or arteritis. Of the mycoplasmas found in man, Mycoplasma pneumoniae is the only well established human pathogen. It is responsible for a variety of respiratory syndromes, of which the most frequently recognized is cold agglutinin-positive atypical pneumonia. Hematologic, neurologic and dermatologic complications of this infection have been noted. M. hominis has been implicated as a causative factor in various febrile complications of pregnancy, such as septic abortion and amnionitis. T-strain mycoplasmas are ubiquitous in the human genitourinary tract, but attempts to link their presence to disease have thus far been unsuccessful. Mycoplasmas also have been associated with neoplastic disease and with rheumatoid arthritis. The validity of these latter findings is unclear, and additional study is needed.*

MYCOPLASMA IS THE GENERIC NAME assigned in 1954 to the micro-organisms previously called pleuropneumonia-like organisms. The first member of this group was isolated in 1898 by Nocard et al[1] and was proved to cause epidemic bovine pleuropneumonia, a disease which decimated herds of cattle in Western Europe during the latter half of the nineteenth century. By 1910, the microscopic morphologic features of the organisms had been described.[2] Despite their propagation in cell-free broth, they were considered viruses until the early 1930's, when they were shown to form very small colonies on serum-containing agar.[3] Due to dense growth in

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the center and light growth at the periphery these colonies appeared under the microscope as small fried eggs. This colonial morphologic feature has become a hallmark of the mycoplasmas (Figure 1).

Confusion arose in 1935, when Klieneberger[4] observed "fried egg" colonies in a culture of the bacterium Streptobacillus moniliformis (a cause of rat-bite fever). In honor of the Lister Institute, the term L-form* was applied to all such organisms.

Until recently, the relationship between L-forms and mycoplasmas has been controversial. Sophisticated techniques of nucleic acid analysis have now suggested that mycoplasmas are a genetically distinct group of micro-organisms.[5,6]

Mycoplasmas do not have a cell wall. Electron micrographs show them to be extremely pleomorphic organisms bounded by a three-layer unit membrane. They are about the same size as the large viruses, with the smallest reproductive units the size of the myxoviruses (about 125 millimicrons).[7]

Much of our knowledge of mycoplasmas derives from study of infections in lower mammals. At least 40 species have been isolated, many of which are pathogenic for one or more animal hosts. A sampling of veterinary diseases caused by mycoplasmas would include pneumonia and pericarditis in cattle,[1] goats[8] and rodents;[9] purulent or chronic relapsing arthritis in swine,[10] rodents[11] and fowl;[12] arteritis in turkeys[13] and encephalitis in mice.[14] Recently, mycoplasmas have been associated with an increasing number of plant diseases; several apparently respond to tetracycline therapy.[15]

Pathogenicity in man was first suggested by the isolation of a mycoplasma in pure culture from a Bartholin's gland abscess in 1937.[16] Since then, seven distinct species and another group of biologically similar but as yet unclassified organisms (the "T" strains) have been isolated regularly from man.[17] Of these, Mycoplasma pneumoniae, a common respiratory pathogen, has satisfiied Koch's postulates; the status of the T-strains, which are found in the genitourinary tract, is uncertain; and M. hominis appears to be an opportunistic genital pathogen. Of the remainder, M. salivarium is frequently found in saliva and gingival scrapings and M. orale 1 is a common inhabitant of the oropharynx; M. orale 2 and 3 are less common inhabitants of the oropharynx, and M. fermentans, though rarely isolated, can be found in either the respiratory or genital tracts. Several animal species also have been recovered from human specimens inoculated into various tissue culture systems. The validity of these isolations is in doubt due to the high rate of tissue culture contamination by mycoplasmas.[18]

*Subsequently, others have used such terms as *protoplast, spheroplast, L-phase growth* and *atypical bacterial variant* to describe cell wall defective organisms derived from a variety of bacterial species.

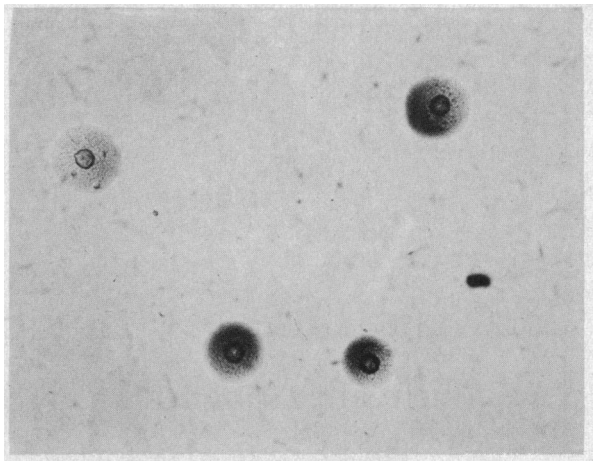

Figure 1.—Typical fried-egg colony produced on agar by mycoplasmas (magnification X20).

Mycoplasma pneumoniae

Atypical pneumonia was noted as an entity distinct from pneumococcal (typical) pneumonia in 1938,[19] but the disease did not assume major importance until World War II. During the early 1940's, epidemics recurred in Armed Forces training centers. Though these epidemics were characterized by low mortality, the two- to three-week duration of illness disrupted training schedules.

In 1941, Peterson, Ham and Finland[20] identified the presence of cold agglutinins in convalescent sera from approximately 50 percent of patients with this syndrome. As a group, the cold agglutinin-positive patients tended to have a more prolonged course. Their filtered, bacteriologically sterile sputum was shown to transmit disease to human volunteers.[21] Further work by Eaton, et al established that the agent could be passed to certain rodents[22] and could be propagated in chick embryo. Liu later demonstrated discrete particulate bodies localized in chick embryo bronchial epithelium;[23] and, in 1961, Marmion and Goodburn suggested that the organism

might be a mycoplasma rather than a virus.[24] One year later, the organism was grown in a cell-free medium by Chanock, Hayflick and Barile[25] and was named Mycoplasma pneumoniae. The artificially cultivated organism has been shown by serological methods to be identical to Eaton's agent and to produce the identical disease in human volunteers.[25,26]

The course of infection by M. pneumoniae is variable, ranging from subclinical through bronchopneumonia to rare cases of lobar pneumonia with effusion.[17] In most cases the disease begins with a prodrome of malaise and upper respiratory symptoms. Severe headache is often a prominent complaint.[27] Shortly after onset a persistent cough which may be productive of small amounts of sputum usually develops. Fever between 38.1° and 38.9° C (100.8° and 102° F) is usual, but the patient's malaise is often greatly out of proportion to the degree of fever. Arthralgias, nausea or vomiting may also be noted. Careful physical examination may disclose only a few rhonchi with fine râles over the posterior mid-lung fields. Radiographically, extensive infiltration—more than would be anticipated from the localized physical findings—may be seen. The superior segment of the lower lobe is involved most frequently. In about 20 percent of some series, the infiltrate is bilateral; occasionally a small pleural effusion may be found.[28,28a]

The leukocyte count is usually normal or mildly elevated with an essentially normal differential. Cold agglutinins are usually not found in the first few days of illness, but if the patient is seen later in the course of the illness they may be present. By the end of the third week, 40 to 80 percent of patients will have elevated cold agglutinins,* and 80 to 90 percent of patients will have an elevated complement fixation titer to M. pneumoniae. On admission, the Coombs test may be positive, especially in the presence of cold agglutinins.[29] Though the organism usually takes from two to three weeks to grow on primary isolation, culturing of material from the oropharynx or sputum is relatively simple and should be done where possible for epidemiological reasons (see appendix). Culture combined with serologic methods should identify close to 100 percent of cases.

M. pneumoniae epidemics are characterized by their slow spread with an incubation period between contacts of about three weeks.[30] The incidence rate of M. pneumoniae infection is highest among adolescents and young adults. Myringitis may occur but is more common in children.[31] Primary atypical pneumonia can affect patients of any age; but, in children below six years and adults between 45 and 70 the disease is rare.[31,32]

Although year to year incidence of M. pneumoniae infection may vary widely in different geographic locations, the seasonal incidence in the general population is fairly uniform.[33] Thus, in winter, when bacterial pneumonia is more common, M. pneumoniae causes a smaller proportion of disease than it does in the summer when overall rates of pneumonia are low.

The total course of the untreated pneumonia is usually about three weeks, with fever persisting for five to seven days and the radiographic infiltrate visible for two to three weeks. On occasion, malaise and cough may be present for as long as two months.[33]

With exception of an occasional case of pericarditis,[34] complications of M. pneumoniae infection are rare. Bacterial superinfection is unusual.[33] Several cases of fatal hemolytic anemia have been reported in association with high titers of cold agglutinins,[35] and Feizi suggested M. pneumoniae infection may be the initial trigger in some cases of chronic "autoimmune" hemolytic anemia.[29] Renal failure in association with high cold agglutinin levels has also been reported.[36] Neurological complications of M. pneumoniae infection can be quite varied. Encephalitis, meningo-encephalitis,[37] transverse myelitis and Guillain-Barré syndrome[38] all have been seen in patients with atypical pneumonia from whom either M. pneumoniae was isolated or in whom serological evidence of infection was demonstrated. Several cases of each have also been reported in patients with no antecedent history of respiratory ailment, but with serological evidence of recent M. pneumoniae infection. Most of the encephalitis cases reported have been self-limited, but deaths have occurred in patients with Guillain-Barré syndrome and transverse myelitis.

Erythema multiforme minor and exudativum (Stevens-Johnson syndrome)[39] both have been associated with M. pneumoniae respiratory infection, and the organism itself has been cultured from blister fluid.[40] Petechial and macular eruptions also have been noted, but these have been

*Though adenovirus pneumonia may also be cold agglutinin-positive, this infection is uncommon in civilian population groups.

transient and of little apparent importance.[28] The erythema multiforme syndromes are significant because of the discomfort they cause and the possibility of serious superinfection. It is unlikely that M. pneumoniae infection causes all Stevens-Johnson-like illness, but the true extent of the association has not been determined. Whether antibiotic therapy of the antecedent respiratory infection will prevent complicating neurological, hematological or dermatological illness is not known.

Three antibiotics have been shown to lessen the morbidity associated with M. pneumoniae infection and to speed resolution of the pulmonary infiltration. These are tetracycline, erythromycin and demethylchlortetracycline.[41,42] Penicillin or penicillin-like drugs have no antimycoplasmal activity because these organisms lack a cell wall. Each of the three drugs is equally effective in treating the clinical illness, and choice of antibiotic should be based on patient tolerance. Therapy may be discontinued 48 to 72 hours after the patient has become afebrile. Although erythromycin is the most active against the organism *in vitro*, tetracycline has been found to be slightly more effective in reducing spread of infection to susceptible contacts; but, as is the case with each of these antibiotics, the organism can still be isolated from the oropharynx of 15 to 20 percent of patients for as long as three months after clinical recovery.[42]

Although M. pneumoniae infection usually cannot be differentiated prospectively from viral pneumonitides, the reduction of morbidity in mycoplasmal disease by antibiotics is sufficient to warrant a recommendation that patients presenting with atypical pneumonia probably should be treated. A sputum smear and other appropriate studies must always be performed to rule out bacterial infection. Penicillin is still the drug of choice for pneumococcal pneumonia.

T-Strain Mycoplasmas

T-strain mycoplasmas were described by Shepard in 1954.[43] These organisms are distinguished by the tiny colonies they form on agar (approximately 10μ to 15μ in diameter) and by their requirement for urea as an energy source.[44] During the past decade, they have been implicated as a cause of non-gonococcal urethritis, post-gonococcal urethritis, female infertility, abortion, "abacterial pyelonephritis" and other obscure diseases of the genitourinary tract. Teleologically, their metabolic requirement for urea makes the T-strains an ideal hypothetical candidate to cause almost any genitourinary disease of an unknown, but possibly infectious, nature. Recently improved cultural techniques have shown their ubiquity in the lower genitourinary tract, to the extent that Braun et al[45] have reported isolation of T-strains from the urine or vagina of 79 percent of women on their initial visit to a routine prenatal clinic. Further, several investigations performed in England comparing isolation rates from urethral swabs in normals to those in patients (male or female) with gonococcal urethritis, non-gonococcal urethritis, post-gonococcal urethritis, trichomonas vaginitis or urethritis and other "non-specific" genital infection have shown no significant differences between any of these groups.[46,47,48] Although Braude showed bladder stone formation in rats after intrarenal injection of T-strains,[49] the organisms have not been isolated from the upper urinary tract of man or animal. Furthermore, no simple serological test for evidence of infection is available to help delineate a pathogenic role for these organisms. Despite these problems, a group of investigators in Boston recently noted the birth weight of infants from Negro mothers who had cultures positive for T-strains before delivery was significantly lower than that of infants from mothers from whom no mycoplasmas were isolated.[50] Whether these data represent cause, effect, or a chance association with other interrelated factors is not yet known.

Mycoplasma hominis

Although T-strains and M. hominis are often found together, evidence that M. hominis might be a human pathogen is stronger than for T-strains. Three tests for serological response to M. hominis are available: complement fixation, indirect hemagglutination and metabolism inhibition. Each of these has been applied to broad population groups with somewhat similar results. In women, antibody begins to appear with puberty; and titers increase steadily through the reproductive years to peak in the fifth decade and then fall after age 50. In men, antibody also appears with puberty but rises to lower levels than in women, increasing steadily with age to the seventh decade. Comparing matched age groups

and sexes, titers to M. hominis are higher in groups with increased sexual promiscuity and low socio-economic status.[51]

These data have suggested that M. hominis might be involved in (1) venereal diseases, (2) infectious complications of the reproductive system in women and (3) in urinary tract problems of older men. No convincing evidence has been marshalled to support propositions one or three, but proposition two has been a more fruitful pursuit: M. hominis has been isolated from Bartholin's gland abscesses,[16] ovarian abscesses and blood cultures of women with ovarian abscesses,[52] blood cultures taken during fever appearing after therapeutic abortion,[53] blood cultures taken during spontaneous septic abortion,[54] blood cultures taken during postpartum fever,[55,56,57] amniotic fluid following vaginal examination, internal organs of an aborted fetus,[54] and from newborns with conjunctivitis.[58] Vaginal or cervical colonization with M. hominis also has been implicated as a cause of prematurity,[59] and higher isolation rates have been found in women in whom fever develops after delivery[60] as well as in women with septic as opposed to afebrile spontaneous abortion. In the last comparison, the proportion of serological response to M. hominis was also higher in febrile patients. Of the 51 patients with abortal sepsis in this series, six were shown to have septicemia which, in four cases, was due to M. hominis.[61] Since none of these four patients was treated with an effective antibiotic (lincomycin or tetracycline are the most active[62]) and all recovered, a strong case cannot be made for routine use of antimycoplasmal antibiotics in these cases. In most of the above situations, evidence supports the role of M. hominis as an opportunistic pathogen, invading when local tissue trauma has produced a conducive environment.

M. hominis has also been shown experimentally to cause exudative pharyngitis in antibody-free human volunteers,[63] but the organism has been isolated from clinical respiratory tract disease so infrequently that it probably should not be considered a respiratory pathogen.[64]

Mycoplasmas and Neoplastic Disease

Reports have appeared in the past decade linking mycoplasmas to human leukemia and lymphoma. These studies were initiated to define a viral cause for human leukemia; thus, when cytopathic effects were seen and passed in tissue culture, the bodies discovered by electron microscopy were assumed to be viruses. By 1963, the first of these agents identified as a mycoplasma (specifically M. pulmonis) had been described. More than likely, it represented a tissue culture contaminant. Subsequently, M. orale 1, M. fermentans, M. laidlawii and M. hominis all have been isolated from tissue culture inoculations of leukemic bone marrow. More recently, M. orale 1 and M. fermentans have also been isolated in artificial medium from bone marrow of leukemics,[65,66] but these isolations have been sporadic and from only a small percentage of patients studied. Murphy et al[67] concluded that these isolations represent the invasion of mucous membranes by the normal mycoplasma flora in an immunologically compromised host.

Mycoplasmas and Arthritis

Since Sabin's description of polyarthritis in mice due to a mycoplasma,[68] at least 12 other mycoplasma species have been shown to cause arthritis in various mammalian hosts under both natural and experimental circumstances.[69] This his stimulated many studies of the etiologic role of mycoplasmas in human rheumatoid arthritis (RA). A variety of organisms have been recovered in tissue culture inoculation of joint specimens, but for the reasons noted above these isolations are all suspect. Williams, using only an artificial growth medium, noted a significant rate of isolation of M. fermentans from synovial fluid of patients with RA.[70] Subsequently Williams and his associates demonstrated delayed hypersensitivity to an M. fermentans antigen in 29 of 43 RA patients.[71] These findings, as yet unconfirmed by other investigators, have renewed interest in the search for an infectious etiologic factor in human RA.

APPENDIX

The common human mycoplasmas are grown easily in a simple medium and can be differentiated tentatively by four chemical tests: glucose oxidation, arginine deamination, urea hydrolysis and methylene blue reduction or growth inhibition (Table 1). Final identification is based upon immunological methods, of which the

TABLE 1.—*Differentiation of Mycoplasmas*

	Methylene Blue Reduction	*Glucose*	*Arginine*	*Urea*
M. pneumoniae	+	+	−	−
M. hominis	No growth	−	+	−
M. fermentans	±	+	±	−
M. orale 1, 2, 3	No growth	−	−	−
M. salivarium	No growth	−	−	−
T-strains	No growth	−	−	+

simplest is the growth inhibition technique of Clyde.*[72]

The basic growth medium is prepared as follows:

Mycoplasma broth base (Difco or BBL, Inc., or other)	70%
Yeast extract (Microbiological Associates or other)	10%
Sterilize, then add:	
Phenol red	0.02%
Horse, human, swine or calf serum	20%
Penicillin G	1,000 units per ml
Polymyxin B	25 ug per ml**

From this basic medium three variations are made:

#1 add 1% glucose and 0.002% w/v methylene blue chloride; adjust pH to 7.6.

#2 add 0.5% w/v arginine; adjust pH to 6.7.

#3 add 0.5% w/v urea; adjust pH to 6.0.

The three media are dispensed in aliquots of 5 ml and frozen until needed. They should remain stable for several months.

Throat swabs for M. penumoniae isolation are inoculated only into the methylene-blue glucose media. As methylene blue will inhibit the other oral mycoplasma,[75] a color change from violet to yellow or yellow-green between eight and 28 days indicates glucose oxidation and reduction of methylene blue, which presumptively identifies M. pneumoniae.

Urine and blood (1 ml) and urethral, vaginal or other swabs from a genitourinary source should be inoculated into all three media (methylene-blue glucose last). Color change in arginine broth (from orange to red) between 24 and 96 hours suggests growth of M. hominis; color change in urea broth (from yellow-orange to red) between 18 and 48 hours suggests growth of a T-strain; after 48 hours, a color change in urea broth could be due to M. hominis. Thus, the urea and arginine media must be compared if the urea broth becomes positive after 48 hours. Last, a color change from violet to yellow-green in the methylene-blue glucose medium between five and eight days would suggest the presence of M. fermentans.

Any positive culture must be subcultured to bacteriological media without antibiotics to rule out a bacterial contaminant as the cause of the color change.

*Antisera for this test are commercially available from several sources.

**Thallium acetate has been omitted from this basic medium because at concentrations required to inhibit Gram-negative bacteria, T-Strain mycoplasmas will also be inhibited.[73] This is unlikely with the use of Polymixin B at this relatively low concentration.[74]

REFERENCES

1. Nocard E, Roux ER, Borrel A, et al: Le microbe de la péripneumonie. Ann Inst Pasteur 12:240-262, 1898

2. Bordet J: La morphologie du microbe de la péripneumonie des bovides. Ann Inst Pasteur 24:161-167, 1910

3. Ledingham JCG: The growth phases of pleuropneumonia and agalactia on liquid and solid media. J Pathol Bacteriol 37:393-410, 1933

4. Klieneberger E: The natural occurrence of pleuropneumonia-like organisms in apparent symbiosis with Streptobacillus moniliformis and other bacteria. J Pathol Bacteriol 40:93-105, 1935

5. Dowell VR, Loper JC, Hill EO: Constancy of DNA base composition in transition of Sphaerophorus necrophorus from bacilli to large bodies. J Bacteriol 88:1805-1807, 1964

6. McGee ZA, Rogul M, Wittler RG: Molecular genetic studies of relationships among mycoplasma, L-forms and bacteria. Ann NY Acad Sci (Art. 1) 143:21-30, 1967

7. Hayflick L, Chanock RM: Mycoplasma species of man. Bacteriol Rev 29:185-221, 1965

8. Hudson JR, Cottew GS, Adler HE: Diseases of goats caused by mycoplasma: A review of the subject with some new findings. Ann NY Acad Sci (Art. 1) 143:287-297, 1967

9. Edward DG *FF*: The occurrence in normal mice of pleuropneumonia-like organisms capable of producing pneumonia. J Pathol Bacteriol 50:409-418, 1940

10. Roberts ED, Switzer WP, Ramsay FK: The pathology of Mycoplasma hyorhinis arthritis produced experimentally in swine. Am J Vet Res 24:19-31, 1963

11. Sabin AB: The filterable micro-organisms of the pleuropneumonia group. Bacteriol Rev 5:1-67, 1941

12. Chalquest RR, Fabricant J: Pleuropneumonia-like organisms associated with synovitis in fowl. Avian Dis 6:36-43, 1960

13. Thomas L, Davidson M, McCluskey RT: Studies of PPLO infection—I. The production of cerebral polyarteritis by Mycoplasma gallisepticum in turkeys; the neurotoxic property of the mycoplasma. J Exp Med 123:897-912, 1966

14. Thomas L, Aleu F, Bitensky W, et al: Studies of PPLO infection —II. The neurotoxin of M. neurolyticum. J Exp Med 124:1067-1082, 1966

15. Whitcomb RF, Davis RE: Mycoplasma and phytarboviruses as plant pathogens persistently transmitted by insects. Annu Rev Entomol 15:405-464, 1970

16. Dienes L, Edsall J: Observations on L-organisms of Klieneberger. Proc Soc Exp Biol Med 36:740-744, 1937

17. Purcell RH, Chanock RM: Role of mycoplasmas in human respiratory disease. Med Clin North Am 51:791-802, 1967

18. Sabin AB: Nature and source of mycoplasma in various tissue cultures. Ann NY Acad Sci (Art. 1) 143:628-634, 1967

19. Reimann HA: Acute infection of respiratory tract with atypical pneumonia; disease entity probably caused by a filtrable virus. JAMA 111:2377-2384, 1938

20. Peterson OL, Ham TH, Finland M: Cold agglutinins in primary atypical pneumonia. Science 97:167-168, 1943

21. Commission on Acute Respiratory Diseases: The transmission of primary atypical pneumonia to human volunteers. Johns Hopkins Med J 79:97-167, 1946

22. Eaton MD, Meiklejohn G, van Herick W: Studies on the etiology of primary atypical pneumonia. A filtrable agent transmissible to cotton rats, hamsters, and chick embryos. J Exp Med 79:649-668, 1944

23. Liu C: Studies on primary atypical pneumonia. I. Localization, isolation, and cultivation of a virus in chick embryos. J Exp Med 106: 455-467, 1961

24. Marmion BP, Goodburn GM: Effect of an organic gold salt on Eaton's primary atypical pneumonia agent and other observations. Nature 189:247-248, 1961

25. Chanock RM, Hayflick L, Barile MF: Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc Natl Acad Sci 48:41-49, 1962

26. Couch TR, Cate MD, Chanock RM: Infection with artificially propagated Eaton agent. (Mycoplasma pneumoniae.) JAMA 187:146-151, 1964

27. Mogabgab WJ: Mycoplasma pneumoniae and adenovirus respiratory illness in military and university personnel, 1959-1966. Am Rev Resp Dis 97:345-358, 1968

28. Lambert HP: Infections caused by Mycoplasma pneumoniae. Br J Dis Chest 63:71-82, 1969

28a. Fine NL, Smith LR, Sheedy PF: Frequency of pleural effusions in mycoplasma and viral pneumonias. N Engl J Med 283:790-793, 1970

29. Feizi T, Maclean H, Somerville RG, et al: Studies on an epidemic of respiratory disease caused by Mycoplasma pneumoniae. Br Med J 1:457-460, 1967

30. Grayston JT, Alexander ER, Kenny GE, et al: Mycoplasma pneumoniae infections. Clinical and epidemiologic studies. JAMA 191: 369-374, 1965

31. Clyde WA, and Denny FW: Mycoplasma infections in childhood. Pediatrics 40:669-684, 1967

32. Mufson MA, Chang V, Gill V, et al: The role of viruses, mycoplasmas and bacteria in acute pneumonia in civilian adults. Am J Epidemiol 86:526-544, 1967

33. Denny FW, Clyde WA, Glezen WP: Mycoplasma pneumoniae disease: Clinical spectrum, pathophysiology, epidemiology, and control. J Infect Dis 123:74-92, 1971

34. Rytel MW: Primary atypical pneumonia: Current concepts. Am J Med Sci 247:84-104, 1964

35. Maisel JC, Babbit LH, John TJ: Fatal Mycoplasma pneumoniae infection with isolation of organisms from lung. JAMA 202:287-290, 1967

36. Lawson DH, Lindsay RM, Sawers JD, et al: Acute renal failure in the cold agglutination syndrome. Lancet 2:704-705, 1968

37. Taylor MJ, Burrow GN, Strauch B, et al: Meningoencephalitis associated with punemonia due to Mycoplasma pneumoniae. JAMA 199:149-152, 1967

38. Steele JC, Gladstone RM, Thanasophon S, et al: Mycoplasma pneumoniae as a determinant of the Guillain-Barré syndrome. Lancet 2:710-714, 1969

39. Katz HI, Wooten JW, Davis RG, et al: Stevens-Johnson syndrome—Report of a case with culturally proven mycoplasma pneumonial infection. JAMA 199:164-166, 1967

40. Lyell A, Gordon AM, Dick HM, et al: Mycoplasma and erythema multiforme. Lancet 2:1116-1118, 1967

41. Kingston JR, Chanock RM, Mufson MA, et al: Eaton agent pneumonia. JAMA 176:118-123, 1961

42. Smith CB, Friedewald WT, Chanock RM: Shedding of mycoplasma pneumoniae after tetracycline and erythromycin therapy. N Engl J Med 276:1172-1175, 1967

43. Shepard MC: The recovery of pleuropneumonia-like organisms from Negro men with and without nongonococcal urethritis. Am J Syph Gonor Vener Dis 38:113-124, 1954

44. Shepard MC: Recovery, propagation and characteristics of T-strain PPLO isolated from human cases of nongonococcal urethritis. Ann NY Acad Sci (Art. 10) 79:397-402, 1960

45. Braun P, Klein JO, Lee YH, et al: Methodologic investigations and prevalence of genital mycoplasmas in pregnancy. J Infect Dis 121: 391-400, 1970

46. Taylor-Robinson D, Addey JP, Hare MJ, et al: Mycoplasmas and "non-specific" genital infection—I. Previous studies and laboratory aspects. Br J Vener Dis 45:265-273, 1969

47. Dunlop, EMC, Hare MJ, Jones BR, et al: Mycoplasmas and "non-specific" genital infection—II. Clinical aspects. Br J Vener Dis 45:274-281, 1969

48. Hare MJ, Dunlop EMC, Taylor-Robinson D: Mycoplasmas and "non-specific" genital infection—III. Post-gonococcal urethritis. A prospective study. Br J Vener Dis 45:282-286, 1969

49. Braude AI: Production of bladder stones by L-forms. Ann NY Acad Sci, (Art. 2) 174:896-902, 1970

50. Braun P, Lee YH, Klein JO, et al: Birth weight and genital mycoplasmas in pregnancy. New Engl J Med 284:167-170, 1971

51. Jones DM, Sequiera PJL: The distribution of complement-fixing antibody and growth-inhibiting antibody to mycoplasma hominis. J Hyg (Camb) 64:441-449, 1966

52. Stokes EJ: Human infection with pleuropneumonia-like organisms. Lancet 1:276-279, 1955

53. Tully JG, Brown MS, Sheagren JN, et al: Septicemia due to Mycoplasma hominis type I. N Engl J Med 273:648-650, 1965

54. Harwick HJ, Iuppa JB, Purcell RH, et al: Mycoplasma hominis septicemia associated with abortion. Am J Obstet Gynecol 99:725-727, 1967

55. Tully JG, Smith LG: Postpartum septicemia with mycoplasma hominis. JAMA 204:827-828, 1968

56. Harwick HJ, Purcell RH, Iuppa JB, et al: Mycoplasma hominis and postpartum febrile complications. Obstet Gynecol 37:765-770, 1971

57. Harwick HJ, Iuppa JB, Fekety FR: Microorganisms and amniotic fluid. Obstet Gynecol 33:256-259, 1969

58. Jones DM, Tobin B: Neonatal eye infections due to Mycoplasma hominis. Br Med J 3:467-468, 1968

59. Klein JO, Buckland D, Finland M: Colonization of newborn infants by mycoplasmas. N Engl J Med 280:1025-1030, 1969

60. Jones DM: Mycoplasma hominis in pregnancy. J Clin Pathol 20:633-635, 1967

61. Harwick HJ, Purcell RH, Iuppa JB, et al: Mycoplasma hominis and abortion. J Infect Dis 121:260-268, 1970

62. Harwick HJ, Fekety FR: The antibiotic susceptibility of Mycoplasma hominis. J Clin Pathol 22:483-485, 1969

63. Mufson MA, Ludwig WM, Purcell RH, et al: Exudative pharyngitis following experimental mycoplasma hominis type I infection. JAMA 192:116-122, 1965

64. Mufson MA: Mycoplasma hominis I in respiratory tract infections. Ann NY Acad Sci (Art. 2) 174:798-808, 1970

65. Barile MF, Bodey GP, Snyder J, et al: Isolation of Mycoplasma orale from leukemic bone marrow and blood by direct culture. J Natl Cancer Inst 36:155-159, 1966

66. Murphy WH, Bullis C, Ertel IJ, et al: Mycoplasma studies of human leukemia. Ann NY Acad Sci (Art. 1) 143:544-556, 1967

67. Murphy WH, Bullis C, Dabich L, et al: Isolation of mycoplasma from leukemic and nonleukemic patients. J Natl Cancer Inst 45:243-251, 1970

68. Sabin AB: Experimental proliferative arthritis in mice produced by filtrable, pleuropneumonia-like microorganisms. Science 89:228-229, 1939

69. Sharp JT, Riggs S: Mycoplasmas and rheumatic disease. Rheumatology 1:51-106, 1967

70. Williams MH: Recovery of mycoplasma from rheumatoid synovial fluid, *In* Duthie JJR, Alexander WRM (Eds): Pfizer Medical Monograph No. 3. Edinburgh, Alexander, 1968

71. Williams MH, Brostoff J, Roitt IM: Possible role of Mycoplasma fermentans in pathogenesis of rheumatoid arthritis. Lancet 2:277-280, 1970

72. Clyde WA: Mycoplasma species identification based upon growth inhibition by specific antisera. J Immunol 92:958-965, 1964

73. Shepard MC: Cultivation and properties of T-strains of mycoplasma associated with nongonococcal urethritis. Ann NY Acad Sci (Art. 1) 143:505-514, 1967

74. Braun P, Klein JO, Kass EH: Susceptibility of genital mycoplasmas to antimicrobial agents. Appl Microbiol 19:62-70, 1970

75. Crawford YE: *In* Panos C (Ed): Mycoplasma and Bacterial L-forms: A Microbial Enigma. Cleveland, World, 1967, p 28